Second Interim Report
August 2014 – June 2015

Contract No. W911NF-14-1-0510

Project title: exploration for the biological control of flowering rush, *Butomus umbellatus*

P. Häfliger, R. Leiner, C. Baan, A. Martins, S. Soukou, D. Sjolie, I. Toševski and H.L. Hinz

CABI
Rue des Grillons 1, CH-2800 Delémont, Switzerland

Tel: ++ 41 32 421 4870
Fax: ++ 41 32 421 4871
Email: P. Häfliger@cabi.org

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JUN 2015	**2nd Interim**	**00-08-2014 to 00-06-2015**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Exploration for the Biological Control of Flowering Rush, Butomus umbellatus	**W911NF-14-1-0510**
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
CABI,Weed Biological Control Research,Rue des Grillons 1,CH-2800 Delemont, Switzerland,	**; 1721-EN-01**
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
Army Engineer Research & Development Center, International Research Office (ERDC-IRO), Unit 4507, APO, AE, 09421	11. SPONSOR/MONITOR'S REPORT NUMBER(S) **1721-EN-01**

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Flowering rush (Butomus umbellatus) is a perennial aquatic plant of European origin that was introduced to North America as an ornamental over 100 years ago. It has developed into an aggressive invader of freshwater systems especially in the midwestern and western states of the USA and western Canada. Since no effective control methods are currently available, a biological control project was initiated in spring 2013 and CABI in Switzerland subcontracted to conduct surveys for natural enemies in the area of origin of flowering rush. This interim report summarizes data collected by CABI in 2014 until June 2015. Sites of flowering rush in northern Germany, where the rhizome-feeding weevil Bagous nodulosus is known to occur were revisited in 2014 and new sites were checked in Hungary, the Slovak Republic and Poland. A total of 236 adults were collected from eight different sites. We continued our efforts to improve rearing success with B. nodulosus. However, we are still experiencing high larval mortality and only a few adults emerged from plants to which larvae or ovipositing females had been transferred. We are currently trying different methods in order to further improve rearing success. Nevertheless we were able to set up 150 adults for overwintering on potted plants in a pond and in plastic cylinders filled with water. Overwintering on potted plants placed in a pond worked very well (80% survival). In 2014, we started setting up host-specificity tests under no-choice conditions with B. nodulosus. Of the ten test plant species offered, none was accepted for egg laying by female weevils, confirming the narrow host range of B. nodulosus. We will continue host-specificity tests in 2015. During a field trip to the Slovak Republic at the end of May 2015, we most probably found the second Bagous species described as monophagous on flowering rush, B. validus. Since this species is extremely rare and nothing is known about its biology we will start by studying its life cycle and try to establish a rearing at CABI in Switzerland. We collected several pupae of the agromyzid fly Phytoliriomyza ornata. Since only one fly emerged, we were not able to work with this species in 2014. We suspected the species to overwinter in the pupal stage. Indeed, a fly emerged from eight overwintered pupae in May 2015. However, only parasitoids or nothing emerged from the remaining pupae. We will continue our efforts in summer 2015.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**8**	

Table of Contents

Abstract

Flowering rush (*Butomus umbellatus*) is a perennial aquatic plant of European origin that was introduced to North America as an ornamental over 100 years ago. It has developed into an aggressive invader of freshwater systems especially in the midwestern and western states of the USA and western Canada. Since no effective control methods are currently available, a biological control project was initiated in spring 2013 and CABI in Switzerland subcontracted to conduct surveys for natural enemies in the area of origin of flowering rush. This interim report summarizes data collected by CABI in 2014 until June 2015.

Sites of flowering rush in northern Germany, where the rhizome-feeding weevil *Bagous nodulosus* is known to occur were revisited in 2014 and new sites were checked in Hungary, the Slovak Republic and Poland. A total of 236 adults were collected from eight different sites. We continued our efforts to improve rearing success with *B. nodulosus*. However, we are still experiencing high larval mortality and only a few adults emerged from plants to which larvae or ovipositing females had been transferred. We are currently trying different methods in order to further improve rearing success. Nevertheless we were able to set up 150 adults for overwintering on potted plants in a pond and in plastic cylinders filled with water. Overwintering on potted plants placed in a pond worked very well (80% survival).

In 2014, we started setting up host-specificity tests under no-choice conditions with *B. nodulosus.* Of the ten test plant species offered, none was accepted for egg laying by female weevils, confirming the narrow host range of *B. nodulosus*. We will continue host-specificity tests in 2015.

During a field trip to the Slovak Republic at the end of May 2015, we most probably found the second *Bagous* species described as monophagous on flowering rush, *B. validus.* Since this species is extremely rare and nothing is known about its biology, we will start by studying its life cycle and try to establish a rearing at CABI in Switzerland.

We collected several pupae of the agromyzid fly *Phytoliriomyza ornata*. Since only one fly emerged, we were not able to work with this species in 2014. We suspected the species to overwinter in the pupal stage. Indeed, a fly emerged from eight overwintered pupae in May 2015. However, only parasitoids or nothing emerged from the remaining pupae. We will continue our efforts in summer 2015.

Bagous nodulosus GYLLENHAL (Coleoptera, Curculionidae)

Biology

The weevil *B. nodulosus* is the most promising potential biological control agent found during the first two years of this project. Although it is considered to be a rare and endangered species (Dieckmann, 1983; Gosik, 2006), we found it with no difficulty on our first field trip to northern Germany in 2013, and then later also in Hungary, the Czech and Slovak Republics. Its preferred habitats are shallow, clear and sun-exposed ponds or channels. It seems that timing of surveys is quite important for finding *B. nodulosus*. Observations made in our rearing colony and in the field suggest that the weevils spend most of their time underwater. In spring (May/June), adults can be found on fine and warm days in large numbers feeding on emergent leaves of flowering rush. In mid-May 2014, we collected 120 adults at two sites in northern Germany, and these represented less than half of the weevils we observed in a subset of the *Butomus* stands. We did not want to collect more adults, because one of the sites is protected and there are still concerns about the weevil being endangered. In August, we found only three adults at the same sites, and none in September. A minimum level of water is obviously important for the weevils. One of the *Butomus* site in a wet meadow in northern Germany was almost completely dried up between May and August, and we did not find any weevils during this period. However, in September, when the meadow was covered again with about 10 cm of water, we were able to collect 26 weevils that were sitting on the leaves 1–3 cm below the surface of the water. The observations made in 2014 confirm our earlier assumption that *B. nodulosus* spends most of its life underwater and the frequency and occurrence of the species is therefore often underestimated.

Plate 1. Adult *Bagous nodulosus.*

Rearing

METHODS Overwintered or field-collected adult *B. nodulosus* were kept in cylinders and provided with cut leaves of flowering rush. Leaves were replaced after 3–5 days and dissected for eggs before disposal. Eggs found were kept in Petri

dishes (diameter 5.5 cm) on moist filter paper until larval hatch. To establish a rearing colony, more than 900 newly emerged larvae were transferred between mid-May and early August onto about 70 potted flowering rush plants covered with gauze bags. An additional 66 adults were kept for oviposition on 17 potted plants covered with gauze bags for 1–3 weeks. Plants were kept submerged to a depth of 3–20 cm in two 4 m × 2 m pools. Plants were dissected for larvae or pupae after 2–4 weeks. Whenever possible, we measured head capsule diameters to determine the larval instar.

RESULTS Weevils collected in mid-May 2014 in northern Germany had stopped ovipositing by the end of May, while weevils collected in June in the Slovak Republic and Hungary continued ovipositing until the end of July. The dissection of the first two plants after 2–3 weeks gave very promising results, i.e. we found 3–4 live individuals from the five larvae transferred. Two had already pupated successfully. However, most of the larvae transferred onto the remaining pots died before or immediately after pupation. We are unsure why this happened, but hypothesize that it might be related to water temperature/quality, bacterial infection or predation. We tried testing different set-ups, keeping plants in shallow or deep water, or in buckets with regularly exchanged water. However, these measures only slightly increased rearing success. The lower than average temperatures in summer 2014 might have negatively influenced development of *B. nodulosus*.

We are currently testing additional set-ups in order to increase rearing success, for instance using different water levels in natural and artificial ponds. Since we observed adult weevils at a site that almost completely dried out in summer 2014, we will also try transferring larvae onto unsubmerged plants.

About 50 adults were overwintered on 12 potted plants covered with gauze bags in an artificial pond, and about 100 adults were set up, like last winter, in stacked plastic cylinders (height 27 cm, diameter 11 cm) filled to a depth of 4–8 cm with water. Overwintering on potted plants placed in a pond worked very well (80% survival), while survival on cylinders was lower than 50%.

Host-specificity tests

From mid-May to mid-June 2014, we established our first host-range trials, set up as sequential no-choice oviposition tests. Since it would require too much material in terms of plants and insects, we did not use potted plants, but tested a set-up using cut leaves instead.

METHODS To ensure that only egg-laying females were used for this test, females were kept individually for 1–2 days on a *Butomus* leaf. Only females that laid more than one egg within two days were used. Cut leaves of test plants were individually exposed to ovipositing females for two days in plastic cylinders (volume 1.3 litres) half-filled with water. Females were then placed onto cut leaves of flowering rush to verify that they were still laying eggs. Tests were only considered valid if the female laid at least one egg on the control (flowering rush) within three days after the test. Females that were still laying eggs were subsequently exposed to another set of test plants. Eggs found during the tests were used to supplement our rearing colony.

RESULTS Using this method, a total of ten test plant species were exposed to *Bagous nodulosus* females between 18 May and 20 June. A few feeding marks were found on some of the test plants, but none of them were accepted for egg laying.

Impact

Between 21 and 27 May 2015, an impact experiment was established by releasing different densities (0, 1, or 3 pairs) of *B. nodulosus* onto individually potted, gauze-covered flowering rush plants. Ten replicates were established per density. Only egg laying females were used. Prior to set up plant size was measured and it was ensured that there were no initial differences in plant parameters between treatments. After two weeks, weevils will be removed from the plants and feeding damage quantified. After another 3-4 weeks, plants will be re-measured and then regularly checked for adult emergence. Results will be available later this year.

Other Species

Based on our literature and field surveys in 2013, we prioritized three additional insects as potential biocontrol agents. All three species are recorded only from flowering rush.

Bagous validus ROSENHAUER (Coleoptera, Curculionidae)

Detailed site records for *B. validus*, a sibling species to *B. nodulosus*, are very rare. The only recently published record is from Serbia (Pesic, 2002). The Hungarian entomologists Otto Merkl, Attila Podlussány and Gábor Hegyessy (Hungarian Natural History Museum, Budapest) sent us the coordinates of a few sites in Hungary where this weevil was found about 20 years ago. However, in 2014, we found flowering rush at only one of these sites, and we did not find *B. validus* there.

Between 28 May and 2 June 2015, a field trip was conducted to the Czech and Slovak Republic together with Petr Bogusch (University of Hradec Králové). At one site in the Slovak Republic we were able to collect 25 individuals of what appears to be *B. validus* in a region, where the species had been recorded in 1975. We will send specimens to a taxonomist for confirmation. Since this species is extremely rare and nothing is known about its biology, we will concentrate on elucidating its life cycle and try establishing a rearing at CABI.

Phytoliriomyza ornata (MEIGEN) (Diptera, Agromyziada)

The genus name of this agromyzid fly (syn. *Metopomyza ornata*) has been changed several times in the past. According to the agromyzid specialist Prof. von Tschirnhaus (Bielefeld, Germany), the current valid genus name *Phytoliriomyza* should be changed back to *Cerodontha*.

We found about 20 pupae of *P. ornata* upon dissection of flowering rush leaves collected in northern Germany in August and September 2014. However, apart from a few parasitoids, only one fly has emerged so far. We are currently keeping half of the pupae in a wooden shelter at ambient temperatures because *P. ornata* may overwinter in the pupal stage.

Plate 2. Pupae and adult of *Phytoliriomyza ornata*.

***Hydrellia concolor* (STENHAMMER) (Diptera, Ephydridae)**

The only four specimens of an ephydrid fly that we were able to rear through or collect as adults in 2013 were sent for identification to Jens-Hermann Stuke (Leer, Germany). However, since they were all females, it was only possible to confirm that they belong to the genus *Hydrellia.* Although there is only one *Hydrellia* species recorded on flowering rush in the literature, we seem to have found two different species of this genus. Unfortunately, we were able to collect only empty *Hydrellia* pupal cases in 2014. We will need to collect flowering rush earlier in 2015 in order to obtain enough males for further identifications.

Plate 3. Larva and adult ~~?~~ *Hydrellia* sp. with empty pupal case.

Acknowledgements

We thank again Henrich Klugkist (Senator für Umwelt, Bau und Verkehr, Bremen, Germany) and Karin Menke (Büro für Landschaftsökologie, Bremen, Germany) for supporting the permit to access the Landschaftschaftschutzgebiet Niedervieland and to collect *Bagous nodulosus*. We are grateful to Otto Merkl, Attila Podlussány and Gábor Hegyessy for sharing site records for *Bagous validus*. Prof. M. von Tschirnhaus (Bielefeld, Germany) kindly gave information about *Phytoliriomyza*

ornata and sent our flies to the Diptera specialist Jens-Hermann Stuke. Many thanks to Jens-Hermann Stuke (Leer, Germany) who examined our *Hydrellia* species. Martin Hanzl (Academy of Science of the Czech Republic) sent information about the ploidy level of *Butomus* populations from the Slovak Republic and the coordinates of their sites. The Teichwirtschaft Götsch in Muxall, Germany, kindly allowed us access to their ponds. We also thank Rafal Gosik (Maria Curie-Sklodowska University, Lublin, Poland), for providing the coordinates of a *Bagous* site in Poland. We are grateful to John Gaskin (USDA-ARS Sidney, Montana) who is conducting the molecular work on *Butomus* populations sampled in Europe and North America. Many thanks also to Daniel Slodovic (University of Fribourg, Switzerland) and Petr Bogusch (University of Hradec Králové for field assistance. Our colleague Tim Haye (CABI Switzerland) took some great pictures of adult *B. nodulosus.* Florence Willemin (CABI Switzerland) propagated and maintained flowering rush and test plants.

Finally, we are very grateful to Jennifer Andreas (Integrated Weed Control Project, Washington State University Extension, USA), Greg Haubrich (Washington Department of Agriculture), Peter Rice (University of Montana) and Jenifer Parson (Washington Department of Ecology) for their continuing support of the project and their efforts to organize shipments of test plant material and flowering rush rhizomes.

Funding for this project in 2014 came from the U.S. Army Corps of Engineers, the Montana Weed Trust Fund through the University of Montana, and the Washington State Department of Agriculture in the USA, and the British Columbia Ministry of Forests, Lands and Natural Resource Operations in Canada.

References

Dieckmann, L. (1983) Beiträge zur Insektenfauna der DDR: Coleoptera - Curculionidae (Tanymecinae, Leptopiinae, Cleoninae, Tanyrhyncinae, Cossoninae, Raymondionminae, Tanysphyrinae). *Beiträge zur Entomologie* 33, 257–381.

Gosik, R. (2006) Description of the larvae and the pupa of *Bagous nodulosus* Gyllenhal in Schoenherr, 1836 (Coleoptera: Curculionidae), with comments on its biology. *Baltic Journal of Coleopterology* 6, 143–150.

Häfliger, P., Medeiros de Souza, N., Freise, J. and Hinz, H. (2014) Biological control of flowering rush, *Butomus umbellatus*. Annual Report 2013. CABI, Delémont, pp. 25.

Pesic, S. (2002) *Bagous validus* Rosenhauer, 1847 – a new weevil (Curculionidae) in Kragujevac valley. *Acta Entomologica Serbica* 7, 41–50.